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(54) Encapsulation and encapsulated products

(57) An encapsulated product comprises microbial capsule having a lipid content of less than 10% by weight and containing a substantial quantity of an organic liquid, optionally with a material dissolved or microdispersed in the organic liquid, not being natural components of the microbe. The microbe may be a yeast having a negligible lipid content. The organic liquids may be alcohols having more than 3 carbon atoms, esters and liquid hydrocarbons. The materials may be dyes or insecticides. The encapsulation may be performed under a slightly elevated temperature while the microbe is in the form of an aqueous paste. Uses of the product include carbonless copy paper and others wherein the active material is released by rupture of the capsule.

SPECIFICATION

Encapsulation and encapsulated products

5 This invention relates to encapsulated products and their production employing microbial capsules.

A method of producing microbially encapsulated materials is proposed in U S Patent Specification 4001480. According to that specification it is essential that the material to be encapsulated is soluble in the natural fat, i.e. lipid, of the microbe and that the microbe should have a very high lipid content of about 40 to 60% by weight.

Another method of producing microbially encapsulated materials is described in European Patent Publication 85805. In that method a grown microbe is treated with a lipid-extending organic liquid substance defined by tests described in the specification and with a material to be encapsulated which is soluble or microdispersible in the lipid-extending substance. Both the lipid-extending substance and the material are retained passively in the microbe. That method is advantageous over the method described in U S Patent Specification 4001480 in that the material to be encapsulated need not be soluble in the microbial lipid and in that the microbe need not have a very high lipid content. However, the publication states that preferably the microbe should have a significant lipid content, particularly at least 10% by weight.

It is now found that materials may be encapsulated by microbes which have less than 10% by weight lipid content and indeed by microbes having negligible or substantially nil lipid content.

According to the present invention there is provided a method of making an encapsulated product comprising contacting a grown microbe having less than 10% by weight lipid content with an organic liquid which is capable of entering the microbe by diffusion through the microbial cell wall without rupturing it, for a contact time being at least until one or more glistening globules of the organic liquid can be observed (microscopically) to be retained passively in the microbe.

According to the present invention also there is provided an encapsulated product comprising a microbial capsule having a lipid content of less than 10% by weight and containing a substantial quantity of an organic liquid, optionally with a material dissolved or microdispersed in the organic liquid, not being natural components of the microbe.

The organic liquid may itself be the material to be encapsulated. Alternatively the organic liquid may be employed as a solvent or dispersant for the material to be encapsulated. In the latter embodiment the microbe may be contacted with a solution or microdispersion of the material in the organic liquid as a single operation or alternatively the microbe may be contacted first with the organic liquid such that the organic liquid is diffused into it and then contacted with the encapsulatable material in the form of a solution or microdispersion in the same or a different organic liquid or in a solvent or dispersant for the material which is

capable of diffusing through the microbial cell wall without rupturing it and preferably which is miscible with the organic liquid already in the microbe but which is not retained passively in the microbe. In any case, the material is retained passively in the microbe.

The organic liquid should be in the liquid state at the temperature at which the microbe is subjected to the encapsulation treatment. It should pass at least one of the tests described for lipid-extending substances in European Patent Publication 85805.

Examples of suitable organic liquids are as follows:-

1. Alcohols having more than three carbon atoms, especially aliphatic alcohols. The alcohols may have one or more hydroxyl groups. Examples of suitable alcohols are primary alcohols, especially those having 4 to 15 carbon atoms, e.g. n-butanol, octanol, decanol and mixtures of long-chain primary alcohols, mainly of 9 to 15 carbon atoms; secondary alcohols e.g. iso-butanol and octan-2-ol; tertiary alcohols e.g. tertiary-butanol; and glycols such as diethylene glycol ($\text{O}(\text{CH}_2\text{CH}_2\text{OH})_2$).

2. Esters, especially those in which the alcohol component has more than two carbon atoms. The esters may be derived from acids having one or more carboxylic groups, for example 2 or 3 carboxylic groups, and from alcohols having one or more hydroxyl groups, for example 2 or 3 hydroxyl groups. The acid and alcohol components may be aromatic or aliphatic. The acid component may be a hydroxycarboxylic acid. Examples of suitable esters are 2-ethylhexyl acetate, di-2-ethylhexyl adipate, di-iso-butyl phthalate, butyl benzyl phthalate, acetyl tributyl citrate, 2,2,4-trimethyl-1,3-pentandiol iso-butyrate, glyceryl triacetate (triacetin), glyceryl tributyrate, di-n-butyl phthalate, butyl acetate, ethylene glycol mono-acetate, butyl glycol acetate and diethylene glycol dibenzoate.

3. Some liquid hydrocarbons, for example aromatic hydrocarbons such as xylene, hydrogenated aromatic hydrocarbons such as that available as "Santosol 340" and the mixture of 40% hydrogenated terphenyls available as "HB 40", and alkylaromatic hydrocarbons such as di-iso-propyl naphthalene.

Mixtures of lipid-extending substances may be employed.

In some cases, particularly when the organic liquid is normally viscous, it may be desirable to thin or dilute the liquid with a thinner such as acetone in order to facilitate the rate of absorption by the microbe and/or the amount of liquid absorbed. Liquids which may benefit from such thinning include "Santosol 340", "HB 40", phthalate esters and the commercially available mixtures of long-chain primary alcohols.

The material to be encapsulated, when other than the organic liquid itself, may be a solid or a liquid and should be soluble or microdispersible in the organic liquid. It is not necessary for the material to be soluble in the microbial lipid.

Examples of encapsulatable materials are dyes (e.g. leuco dyes), insecticides, herbicides, fungicides, molluscides, nematocides, rodenticides, phero-

mones (e g *Dacus oleae*), insect repellents, insect- and plant-growth regulators, adhesives, adhesive components, odiferous materials (e g perfumes), flavourants, pharmaceuticals and chemicals (e g xylene, ethyl phthalate or kerosene). By way of example only, suitable dyes include Sudan Green, Sudan Blue, Ethyl Eosin, Crystal Violet Lactone, Methylene Blue Lactone, Malachite Green Lactone, Benzoyl Leuco Methylene Blue and MHPTS (michlene hydro para-toluene sulphonate), and suitable insecticides include organo-chlorine compounds such as DDT, organo-phosphorus compounds such as malathion and diazinon, carbamate compounds such as pirimicarb and carbaryl, and synthetic pyrethroid compounds such as permethrin and resmethrin.

Two or more reactive or complementary components may be encapsulated by separate microbial cells, for example reactive adhesive components or complementary dyes.

The microbe is a grown microbe, i e a microbe which has been harvested from its culture medium. A preferred microbe is a fungus, especially a yeast. Preferably the microbe has a large cell size, e g of 5 to 20 microns diameter. By way of example only, the microbe may be brewers' yeast, bakers' yeast or *Candida utilis*. Other yeasts having lipid contents of less than 10% by weight (dry) may be employed.

By "lipid content" as used herein there is meant the total natural lipid in the microbe, i e the total of the structural and storage lipid in the cytoplasm and cell wall.

When the microbe is treated with the organic liquid and the material to be encapsulated, it should be intact, i e not lysed. Usually the treatment will comprise stirring the microbe in the liquid and material. The microbe may be in the form of a paste or slurry in water but a preferred technique comprises employing the microbe in a merely moist form. Preferably the treatment is performed at a slightly elevated temperature, e g 40 to 50°C, which in some cases increases microbial absorption of the organic liquid. An elevated temperature may also increase the amount of encapsulatable material dissolved in the organic liquid. Usually the treatment time should be at least 2 hours, more usually at least 3 hours, to achieve optimum degree of diffusion with the production of one or more glistening globules of organic liquid in the microbial cytoplasm as may be observed by microscopic examination.

If desired, the microbial capsule may be softened or hardened by appropriate treatment. For instance the microbe may be treated with a softening agent such as a proteolytic enzyme, either before or after absorption of the organic liquid and the material to be encapsulated. Such softening may be performed to make the absorption easier and/or to facilitate rupture of the capsule when and if desired. Alternatively the cell wall may be treated with a hardening agent such as an aldehyde, for instance when an increase in the pressure-resistance of the capsule is desired.

The encapsulated material may find application

as a free-standing product or adhered to a substrate.

An example of a use of this invention is in the production of "carbonless" copy paper in which a coating of capsules containing a dye is adhered to one side of a sheet of paper, so that when the paper is subjected to pressure, e g by the type-face of a typewriter or by a manual writing implement, the print will be duplicated on a paper sheet in contact with the coating. Usually when encapsulated dyes produced according to the present invention are applied in the form of an aqueous suspension to the paper, the capsules are capable of adhering to the paper satisfactorily without the assistance of a binder or adhesive. The encapsulated material may be a material which produces a strong colour only when it reacts with another material, which may itself be encapsulated or may be in the form of a non-encapsulated coating on the paper. For instance, the encapsulated material may be a leuco dye which is oxidisable by an acidic material such as an acidic clay to produce the desired print colour. It is preferred that one side of the paper sheet has a coating of the encapsulated material and the other side has an acidic clay coating, so that several sheets can be assembled with the clay-coated face of one sheet in contact with the capsule-coated face of the adjacent sheet. Alternatively a mixed coating of capsules and clay may be employed.

In some cases it may be desirable to employ more than one encapsulated dye in order to attain a desired colour.

Another, advantageous, embodiment of the present invention is in the production of encapsulated insecticides. Such encapsulated insecticides, especially those encapsulated by yeasts, often are found to be more stable and more attractive to insects than non-encapsulated insecticides or insecticides encapsulated in synthetic substances.

A further use of capsules of this invention is in conditions such that the release of the encapsulated material is delayed or prolonged by a slow or gradual lysis of the microbial cells. This could be advantageous for the administration of pharmaceuticals.

The invention is illustrated in the following Examples.

In the Examples, the lipid contents of the yeasts were determined by the following procedure. Approximately 0.5 g of freeze-dried cells were weighed accurately in a conical flask and then mixed with 50 ml 2:1 (v/v) chloroform:methanol. After stirring for 6 hours in an orbital shaker, the mixture was filtered through a Whatman No. 1 filter paper and the filtrate was transferred to a separating funnel with 15 ml of 0.9% aqueous NaCl. The chloroform layer was separated and the remaining mixture was washed twice with 10 ml of chloroform. The washings were combined, evaporated to dryness, and the lipid extract was dissolved in 10 ml diethyl ether. Any insoluble material was filtered off and the filtrate was collected in a pre-weighed beaker and evaporated to dryness. The beaker was then re-weighed to deter-

mine the weight of the lipid.

Example I

Pressed industrial lager yeast obtained from
5 Davenports brewery, Birmingham, was washed
with distilled water and separated by centrifugation
at 2000 r.p.m. for 5 minutes.

The lipid content of the yeast was found to be
about 5.5% based on dry weight.

10 Centrifugal yeast product (aqueous paste) con-
taining 5 g (dry) of the yeast was mixed with 5 g of
a 2% (w/w) solution of crystal violet lactone in di-
iso- propyl naphthalene for 24 hours at 40°C in a
temperature- controlled mixing vessel. The product
15 was harvested by centrifuging at 2000 r.p.m. for 15
minutes and then applied to the opposite side of a
clay-coated paper using K-Bar No.2 supplied by
R.K. Print-Coat Instruments Ltd., U K. The quantity
of capsules applied to the paper was of the order
20 of 1 to 6 g.m.⁻¹.

The paper was dried and tested for duplication
using a standard office typewriter. The result was
at least as good as that of commercially available
carbonless copy paper.

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Example II

4 g (dry weight) of bakers' yeast having a lipid
content of about 4.2% based on dry weight, in the
form of a 20% (w/v) aqueous slurry, were mixed
30 with 4 g of a 2.5% (w/v) di-iso-propyl phthalate so-
lution of a 1.5:1 by weight blend of MHPTS (mich-
lene hydro para- toluene sulphonate) available as
Pergascript Blue 1-3R and BLMB (benzoyl leuco
methylene blue) available as Pergascript Blue S-
35 4G, for 16 hours at 40°C in a temperature-con-
trolled mixing vessel.

The product was harvested, applied to clay-
coated paper and tested as described in Example I
except that the quantity of capsules applied to the
40 paper was of the order of 2 to 5 g.m.⁻¹. The result
was a satisfactory carbonless copy paper.

Example III

A strain of *Candida utilis* grown on a confection-
45 ery effluent and having a lipid content of about
9.8% based on dry weight was harvested by cen-
trifugation and 10 g of an aqueous slurry contain-
ing 2 g (dry) of the yeast was mixed with 2 g of a
3% (W/v) solution of MHPTS, crystal violet lactone
50 and BLMB (1:1:1) in di-iso-butyl phthalate and ace-
tone (80:20), for 16 hours at 40°C in a temperature-
controlled mixing vessel.

The product was harvested, applied to clay-
coated paper and tested as described in Example
55 II. The result was similar to that of Example II.

Example IV

The procedure of Example I was repeated except
that the following organic liquids were employed
60 individually in place of the di-iso-propyl naphthal-
ene, viz. di-*n*-butyl phthalate, di-iso-butyl phthalate,
2-ethyl hexyl acetate, butyl acetate, Dobanol 25 (a
commercially available mixture of higher primary
mono-alcohols), Santosol 340, HB 40, ethylene gly-
65 col monoacetate, butyl glycol acetate, Dobane JN

(detergent alkylate) and Benzoflex 2-45 (diethylene
glycol dibenzoate).

The typewriter test showed a satisfactory car-
bonless copy paper.

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Example V

The procedure of Example I was repeated except
that the concentration of the crystal violet lactone
75 solution was varied, viz. 3, 4, 5, 6, 8, 9 and 10% (w/
v) solutions.

The typewriter test indicated that the 8% solution
gave the best result.

Example VI

80 The procedure of Example I was repeated except
that the contact time of the microbial slurry with
the crystal violet lactone solution was varied, viz. 1,
2, 4, 6, 8, 10, 16, 24, 40, 48 and 64 hours.

The typewriter test indicated that the 24 hour
85 contact time gave the best result.

Example VII

The procedure of Example I was repeated except
that the following quantities of crystal violet lac-
90 tone solution were employed individually, viz. 0.5,
0.75, 1.0 1.5 and 2.0 g/g dry yeast.

The typewriter test showed that the 0.75 and 1.0
g/g quantities gave the best results.

Example VIII

25 g of an aqueous slurry containing 4.9 g (dry)
of the yeast described in Example I were mixed
with 5 g xylene in a manner similar to that de-
scribed in Example I. The product was harvested
100 as described in Example I.

Microscopic examination showed a large glisten-
ing globule occupying the whole of the microbial
cytoplasm.

Example IX

20 g of an aqueous slurry containing 4.51 g (dry)
of the yeast described in Example I were mixed
with 4.5 g of a 50% (w/v) solution of *Dacus oleae*
(pheromone) in xylene for 16 hours at 40°C in a
110 temperature-controlled mixing vessel. The product
was harvested as described in Example I.

Microscopic examination showed a large globule
occupying the whole of the yeast cell. Air-drying
and then crushing the capsules resulted in the
115 characteristic odour of pheromone.

Example X

40 g of an aqueous slurry containing 7 g (dry) of
the yeast described in Example I were mixed with
7 g of a 10% (w/w) solution of DDT (dichloro-di-
phenyl-trichloro- ethane) in xylene for 16 hours at
45°C in a temperature- controlled mixing vessel.
The product was harvested as described in Exam-
ple I.

125 A sample of the product was freeze-dried to
weaken and rupture the capsules and was then
subjected to extraction using xylene. Microscopic
examination of the xylene extract showed large
crystals of DDT. A further sample of the product
130 was tested for active DDT by a bio- assay tech-

nique which indicated that the encapsulated DDT would function satisfactorily.

Example XI

5 24 g of an aqueous slurry containing 4.7 g (dry) of the yeast described in Example I were mixed with 3.7 g of an 80% (w/w) solution of malathion (about 95% active) in xylene for 3 hours at 45°C in a temperature-controlled mixing vessel. The prod-

10 uct was harvested as described in Example I. Microscopic examination of the product showed a large globule occupying the whole of the yeast cell. Chemical analysis of the product showed the presence of active insecticide.

Example XII

22.9 g of an aqueous slurry containing 4.3 g (dry) of the yeast described in Example I were mixed with 3.4 g of a 50% (w/w) solution of pirimicarb in xylene for 4 hours at 50°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination of the product showed several globules occupying approximately 80% of the yeast cell. Examination by bio-assay and chemical analyses indicated the presence of encapsulated active insecticide.

Example XIII

30 9.8 g of an aqueous slurry containing 2.1 g (dry) of the yeast described in Example I were mixed with 1.56 g of a 30% (w/w) solution of permethrin in xylene for 16 hours at 45°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination showed several small globules occupying approximately 60% of the yeast cell.

Example XIV

24.0 g of an aqueous slurry containing 4.56 g (dry) of the yeast described in Example I were mixed with 3.65 g of an 80% (w/w) solution of diazinon in xylene for 4 hours at 50°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination showed a large globule occupying the whole of the yeast cell.

Example XV

21.55 g of an aqueous slurry containing 4.4 g (dry) of the yeast described in Example I were mixed with 3.53 g of a 30% (w/w) solution of carbaryl (about 85% active) in xylene for 6 hours at 45°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination showed a large globule in the yeast cell.

Example XVI

24.6 g of an aqueous slurry containing 4.6 g (dry) of the yeast described in Example I were mixed with 3.74 g of a 50% (w/w) solution of resmethrin (about 45% active) in xylene for 6 hours at 45°C in

a temperature-controlled reaction vessel. The product was harvested as described in Example I.

Microscopic examination showed a similar product to that of Example XIII (permethrin).

CLAIMS

1. An encapsulated product comprising a microbial capsule having a lipid content of less than 10% by weight and containing a substantial quantity of an organic liquid, optionally with a material dissolved or microdispersed in the organic liquid, not being natural components of the microbe.

2. An encapsulated product according to claim 1 wherein the microbe has a negligible or substantially nil lipid content.

3. An encapsulated product according to claim 1 or 2 wherein the microbe is a fungus.

4. An encapsulated product according to any of the preceding claims wherein the microbe is a yeast.

5. An encapsulated product according to any of the preceding claims wherein the microbe has a cell size in the range of 5 to 20 microns diameter.

6. An encapsulated product according to any of the preceding claims wherein the microbe is brewers' yeast, bakers' yeast or *Candida utilis*.

7. An encapsulated product according to any of the preceding claims wherein the organic liquid comprises an alcohol having more than 3 carbon atoms.

8. An encapsulated product according to any of the preceding claims wherein the organic liquid comprises an ester.

9. An encapsulated product according to any of the preceding claims wherein the organic liquid comprises a liquid hydrocarbon.

10. An encapsulated product according to any of the preceding claims wherein the organic liquid has dissolved or microdispersed therein one or more dyes suitable for use in carbonless copy paper capsules.

11. An encapsulated product according to any of claims 1 to 9 wherein the organic liquid has dissolved or microdispersed therein an insecticide.

12. An encapsulated product according to claim 11 wherein the insecticide is selected from organochlorine compounds, organo-phosphorus compounds, carbamate compounds and synthetic pyrethroid compounds.

13. An encapsulated product according to claim 1 and substantially as described in any of the foregoing Examples.

14. An encapsulated product according to claim 1 and substantially as described herein.

15. A method of making an encapsulated product comprising contacting a grown microbe having less than 10% by weight lipid content with an organic liquid which is capable of entering the microbe by diffusion through the microbial cell wall without rupturing it, for a contact time being at least until 1 or more glistening globules of the organic liquid can be observed to be retained passively in the microbe.

16. A method according to claim 15 comprising

stirring the microbe with the liquid at a slightly elevated temperature.

17. A method according to claim 16 wherein the temperature is in the range 40°C to 50°C.

5 18. A method according to any of claims 15 to 17 wherein the contact time is at least 2 hours.

19. A method according to any of claims 15 to 18 wherein the microbe is in the form of an aqueous paste or slurry.

10 20. A method according to any of claims 15 to 18 wherein the microbe is in merely moist form.

21. A method according to any of claims 15 to 20 wherein the microbe is treated with a softening agent such as a proteolytic enzyme to soften the
15 cell wall, before or after absorption of the organic liquid.

22. A method according to any of claims 15 to 20 wherein the microbe is treated with a hardening agent such as an aldehyde to harden the cell wall,
20 after absorption of the organic liquid.

23. A method according to claim 15 and substantially as described in any of the foregoing Examples.

24. A method according to claim 15 and sub-
25 stantially as described herein.

GB2162147

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Encapsulation and encapsulated products

Abstract:

Abstract of GB2162147

An encapsulated product comprises microbial capsule having a lipid content of less than 10% by weight and containing a substantial quantity of an organic liquid, optionally with a material dissolved or microdispersed in the organic liquid, not being natural components of the microbe. The microbe may be a yeast having a negligible lipid content. The organic liquids may be alcohols having more than 3 carbon atoms, esters and liquid hydrocarbons. The materials may be dyes or insecticides. The encapsulation may be performed under a slightly elevated temperature while the microbe is in the form of an aqueous paste. Uses of the product include carbonless copy paper and others wherein the active material is released by rupture of the capsule. Data supplied from the esp@cenet database - Worldwide

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<div>(71) Applicant Dunlop Limited (United Kingdom), 2 Parade, Sutton Coldfield, West Midlands B72 1PF</div> <div>(72) Inventor Nahida Abid Pannell</div> <div>(74) Agent and/or Address for Service J. H. Moore, Dunlop Ltd, PO Box 504, Erdington, Birmingham B24 9QH</div>	<div>(56) Documents cited GB 1364815 EP A1 0085805</div> <div>(58) Field of search B8C</div>

(54) Encapsulation and encapsulated products

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SPECIFICATION

Encapsulation and encapsulated products

5 This invention relates to encapsulated products and their production employing microbial capsules.

A method of producing microbially encapsulated materials is proposed in U S Patent Specification 4001480. According to that specification it is essential that the material to be encapsulated is soluble in the natural fat, i.e. lipid, of the microbe and that the microbe should have a very high lipid content of about 40 to 60% by weight.

Another method of producing microbially encapsulated materials is described in European Patent Publication 85805. In that method a grown microbe is treated with a lipid-extending organic liquid substance defined by tests described in the specification and with a material to be encapsulated which is soluble or microdispersible in the lipid-extending substance. Both the lipid-extending substance and the material are retained passively in the microbe. That method is advantageous over the method described in U S Patent Specification 4001480 in that the material to be encapsulated need not be soluble in the microbial lipid and in that the microbe need not have a very high lipid content. However, the publication states that preferably the microbe should have a significant lipid content, particularly at least 10% by weight.

It is now found that materials may be encapsulated by microbes which have less than 10% by weight lipid content and indeed by microbes having negligible or substantially nil lipid content.

According to the present invention there is provided a method of making an encapsulated product comprising contacting a grown microbe having less than 10% by weight lipid content with an organic liquid which is capable of entering the microbe by diffusion through the microbial cell wall without rupturing it, for a contact time being at least until one or more glistening globules of the organic liquid can be observed (microscopically) to be retained passively in the microbe.

According to the present invention also there is provided an encapsulated product comprising a microbial capsule having a lipid content of less than 10% by weight and containing a substantial quantity of an organic liquid, optionally with a material dissolved or microdispersed in the organic liquid, not being natural components of the microbe.

The organic liquid may itself be the material to be encapsulated. Alternatively the organic liquid may be employed as a solvent or dispersant for the material to be encapsulated. In the latter embodiment the microbe may be contacted with a solution or microdispersion of the material in the organic liquid as a single operation or alternatively the microbe may be contacted first with the organic liquid such that the organic liquid is diffused into it and then contacted with the encapsulatable material in the form of a solution or microdispersion in the same or a different organic liquid or in a solvent or dispersant for the material which is

capable of diffusing through the microbial cell wall without rupturing it and preferably which is miscible with the organic liquid already in the microbe but which is not retained passively in the microbe. In any case, the material is retained passively in the microbe.

The organic liquid should be in the liquid state at the temperature at which the microbe is subjected to the encapsulation treatment. It should pass at least one of the tests described for lipid-extending substances in European Patent Publication 85805.

Examples of suitable organic liquids are as follows:-

1. Alcohols having more than three carbon atoms, especially aliphatic alcohols. The alcohols may have one or more hydroxyl groups. Examples of suitable alcohols are primary alcohols, especially those having 4 to 15 carbon atoms, e.g. n-butanol, octanol, decanol and mixtures of long-chain primary alcohols, mainly of 9 to 15 carbon atoms; secondary alcohols e.g. iso-butanol and octan-2-ol; tertiary alcohols e.g. tertiary-butanol; and glycols such as diethylene glycol ($O(CH_2CH_2OH)_2$).

2. Esters, especially those in which the alcohol component has more than two carbon atoms. The esters may be derived from acids having one or more carboxylic groups, for example 2 or 3 carboxylic groups, and from alcohols having one or more hydroxyl groups, for example 2 or 3 hydroxyl groups. The acid and alcohol components may be aromatic or aliphatic. The acid component may be a hydroxycarboxylic acid. Examples of suitable esters are 2-ethylhexyl acetate, di-2-ethylhexyl adipate, di-iso-butyl phthalate, butyl benzyl phthalate, acetyl tributyl citrate, 2,2,4-trimethyl-1,3-pentane-diol iso-butyrate, glyceryl triacetate (triacetin), glyceryl tributyrate, di-n-butyl phthalate, butyl acetate, ethylene glycol mono-acetate, butyl glycol acetate and diethylene glycol dibenzoate.

3. Some liquid hydrocarbons, for example aromatic hydrocarbons such as xylene, hydrogenated aromatic hydrocarbons such as that available as "Santosol 340" and the mixture of 40% hydrogenated terphenyls available as "HB 40", and alkylaromatic hydrocarbons such as di-iso-propyl naphthalene.

Mixtures of lipid-extending substances may be employed.

In some cases, particularly when the organic liquid is normally viscous, it may be desirable to thin or dilute the liquid with a thinner such as acetone in order to facilitate the rate of absorption by the microbe and/or the amount of liquid absorbed. Liquids which may benefit from such thinning include "Santosol 340", "HB 40", phthalate esters and the commercially available mixtures of long-chain primary alcohols.

The material to be encapsulated, when other than the organic liquid itself, may be a solid or a liquid and should be soluble or microdispersible in the organic liquid. It is not necessary for the material to be soluble in the microbial lipid.

Examples of encapsulatable materials are dyes (e.g. leuco dyes), insecticides, herbicides, fungicides, molluscides, nematocides, rodenticides, phero-

mones (e g *Dacus oleae*), insect repellents, insect- and plant-growth regulators, adhesives, adhesive components, odiferous materials (e g perfumes), flavourants, pharmaceuticals and chemicals (e g xylene, ethyl phthalate or kerosene). By way of example only, suitable dyes include Sudan Green, Sudan Blue, Ethyl Eosin, Crystal Violet Lactone, Methylene Blue Lactone, Malachite Green Lactone, Benzoyl Leuco Methylene Blue and MHPTS (michlene hydro para-toluene sulphonate), and suitable insecticides include organo-chlorine compounds such as DDT, organo-phosphorus compounds such as malathion and diazinon, carbamate compounds such as pirimicarb and carbaryl, and synthetic pyrethroid compounds such as permethrin and resmethrin.

Two or more reactive or complementary components may be encapsulated by separate microbial cells, for example reactive adhesive components or complementary dyes.

The microbe is a grown microbe, i e a microbe which has been harvested from its culture medium. A preferred microbe is a fungus, especially a yeast. Preferably the microbe has a large cell size, e g of 5 to 20 microns diameter. By way of example only, the microbe may be brewers' yeast, bakers' yeast or *Candida utilis*. Other yeasts having lipid contents of less than 10% by weight (dry) may be employed.

By "lipid content" as used herein there is meant the total natural lipid in the microbe, i e the total of the structural and storage lipid in the cytoplasm and cell wall.

When the microbe is treated with the organic liquid and the material to be encapsulated, it should be intact, i e not lysed. Usually the treatment will comprise stirring the microbe in the liquid and material. The microbe may be in the form of a paste or slurry in water but a preferred technique comprises employing the microbe in a merely moist form. Preferably the treatment is performed at a slightly elevated temperature, e g 40 to 50°C, which in some cases increases microbial absorption of the organic liquid. An elevated temperature may also increase the amount of encapsulatable material dissolved in the organic liquid. Usually the treatment time should be at least 2 hours, more usually at least 3 hours, to achieve optimum degree of diffusion with the production of one or more glistening globules of organic liquid in the microbial cytoplasm as may be observed by microscopic examination.

If desired, the microbial capsule may be softened or hardened by appropriate treatment. For instance the microbe may be treated with a softening agent such as a proteolytic enzyme, either before or after absorption of the organic liquid and the material to be encapsulated. Such softening may be performed to make the absorption easier and/or to facilitate rupture of the capsule when and if desired. Alternatively the cell wall may be treated with a hardening agent such as an aldehyde, for instance when an increase in the pressure-resistance of the capsule is desired.

The encapsulated material may find application

as a free-standing product or adhered to a substrate.

An example of a use of this invention is in the production of "carbonless" copy paper in which a coating of capsules containing a dye is adhered to one side of a sheet of paper, so that when the paper is subjected to pressure, e g by the type-face of a typewriter or by a manual writing implement, the print will be duplicated on a paper sheet in contact with the coating. Usually when encapsulated dyes produced according to the present invention are applied in the form of an aqueous suspension to the paper, the capsules are capable of adhering to the paper satisfactorily without the assistance of a binder or adhesive. The encapsulated material may be a material which produces a strong colour only when it reacts with another material, which may itself be encapsulated or may be in the form of a non-encapsulated coating on the paper. For instance, the encapsulated material may be a leuco dye which is oxidisable by an acidic material such as an acidic clay to produce the desired print colour. It is preferred that one side of the paper sheet has a coating of the encapsulated material and the other side has an acidic clay coating, so that several sheets can be assembled with the clay-coated face of one sheet in contact with the capsule-coated face of the adjacent sheet. Alternatively a mixed coating of capsules and clay may be employed.

In some cases it may be desirable to employ more than one encapsulated dye in order to attain a desired colour.

Another, advantageous, embodiment of the present invention is in the production of encapsulated insecticides. Such encapsulated insecticides, especially those encapsulated by yeasts, often are found to be more stable and more attractive to insects than non-encapsulated insecticides or insecticides encapsulated in synthetic substances.

A further use of capsules of this invention is in conditions such that the release of the encapsulated material is delayed or prolonged by a slow or gradual lysis of the microbial cells. This could be advantageous for the administration of pharmaceuticals.

The invention is illustrated in the following Examples.

In the Examples, the lipid contents of the yeasts were determined by the following procedure. Approximately 0.5 g of freeze-dried cells were weighed accurately in a conical flask and then mixed with 50 ml 2:1 (v/v) chloroform:methanol. After stirring for 6 hours in an orbital shaker, the mixture was filtered through a Whatman No. 1 filter paper and the filtrate was transferred to a separating funnel with 15 ml of 0.9% aqueous NaCl. The chloroform layer was separated and the remaining mixture was washed twice with 10 ml of chloroform. The washings were combined, evaporated to dryness, and the lipid extract was dissolved in 10 ml diethyl ether. Any insoluble material was filtered off and the filtrate was collected in a pre-weighed beaker and evaporated to dryness. The beaker was then re-weighed to deter-

mine the weight of the lipid.

Example I

Pressed industrial lager yeast obtained from Davenports brewery, Birmingham, was washed with distilled water and separated by centrifugation at 2000 r.p.m. for 5 minutes.

The lipid content of the yeast was found to be about 5.5% based on dry weight.

Centrifugal yeast product (aqueous paste) containing 5 g (dry) of the yeast was mixed with 5 g of a 2% (w/w) solution of crystal violet lactone in di-iso-propyl naphthalene for 24 hours at 40°C in a temperature-controlled mixing vessel. The product was harvested by centrifuging at 2000 r.p.m. for 15 minutes and then applied to the opposite side of a clay-coated paper using K-Bar No.2 supplied by R.K. Print-Coat Instruments Ltd., U.K. The quantity of capsules applied to the paper was of the order of 1 to 6 g.m.⁻¹.

The paper was dried and tested for duplication using a standard office typewriter. The result was at least as good as that of commercially available carbonless copy paper.

Example II

4 g (dry weight) of bakers' yeast having a lipid content of about 4.2% based on dry weight, in the form of a 20% (w/v) aqueous slurry, were mixed with 4 g of a 2.5% (w/v) di-iso-propyl phthalate solution of a 1.5:1 by weight blend of MHPTS (michlene hydro para-toluene sulphonate) available as Pergascript Blue 1-3R and BLMB (benzoyl leuco methylene blue) available as Pergascript Blue S-4G, for 16 hours at 40°C in a temperature-controlled mixing vessel.

The product was harvested, applied to clay-coated paper and tested as described in Example I except that the quantity of capsules applied to the paper was of the order of 2 to 5 g.m.⁻¹. The result was a satisfactory carbonless copy paper.

Example III

A strain of *Candida utilis* grown on a confectionery effluent and having a lipid content of about 9.8% based on dry weight was harvested by centrifugation and 10 g of an aqueous slurry containing 2 g (dry) of the yeast was mixed with 2 g of a 3% (w/v) solution of MHPTS, crystal violet lactone and BLMB (1:1:1) in di-iso-butyl phthalate and acetone (80:20), for 16 hours at 40°C in a temperature-controlled mixing vessel.

The product was harvested, applied to clay-coated paper and tested as described in Example II. The result was similar to that of Example II.

Example IV

The procedure of Example I was repeated except that the following organic liquids were employed individually in place of the di-iso-propyl naphthalene, viz. di-n-butyl phthalate, di-iso-butyl phthalate, 2-ethyl hexyl acetate, butyl acetate, Dobanol 25 (a commercially available mixture of higher primary mono-alcohols), Santosol 340, HB 40, ethylene glycol monoacetate, butyl glycol acetate, Dobane JN

(detergent alkylate) and Benzoflex 2-45 (diethylene glycol dibenzoate).

The typewriter test showed a satisfactory carbonless copy paper.

Example V

The procedure of Example I was repeated except that the concentration of the crystal violet lactone solution was varied, viz. 3, 4, 5, 6, 8, 9 and 10% (w/v) solutions.

The typewriter test indicated that the 8% solution gave the best result.

Example VI

The procedure of Example I was repeated except that the contact time of the microbial slurry with the crystal violet lactone solution was varied, viz. 1, 2, 4, 6, 8, 10, 16, 24, 40, 48 and 64 hours.

The typewriter test indicated that the 24 hour contact time gave the best result.

Example VII

The procedure of Example I was repeated except that the following quantities of crystal violet lactone solution were employed individually, viz. 0.5, 0.75, 1.0, 1.5 and 2.0 g/g dry yeast.

The typewriter test showed that the 0.75 and 1.0 g/g quantities gave the best results.

Example VIII

25 g of an aqueous slurry containing 4.9 g (dry) of the yeast described in Example I were mixed with 5 g xylene in a manner similar to that described in Example I. The product was harvested as described in Example I.

Microscopic examination showed a large glistening globule occupying the whole of the microbial cytoplasm.

Example IX

20 g of an aqueous slurry containing 4.51 g (dry) of the yeast described in Example I were mixed with 4.5 g of a 50% (w/v) solution of *Dacus oleae* (pheromone) in xylene for 16 hours at 40°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination showed a large globule occupying the whole of the yeast cell. Air-drying and then crushing the capsules resulted in the characteristic odour of pheromone.

Example X

40 g of an aqueous slurry containing 7 g (dry) of the yeast described in Example I were mixed with 7 g of a 10% (w/w) solution of DDT (dichloro-diphenyl-trichloro-ethane) in xylene for 16 hours at 45°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

A sample of the product was freeze-dried to weaken and rupture the capsules and was then subjected to extraction using xylene. Microscopic examination of the xylene extract showed large crystals of DDT. A further sample of the product was tested for active DDT by a bio-assay tech-

nique which indicated that the encapsulated DDT would function satisfactorily.

Example XI

5 24 g of an aqueous slurry containing 4.7 g (dry) of the yeast described in Example I were mixed with 3.7 g of an 80% (w/w) solution of malathion (about 95% active) in xylene for 3 hours at 45°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination of the product showed a large globule occupying the whole of the yeast cell. Chemical analysis of the product showed the presence of active insecticide.

Example XII

22.9 g of an aqueous slurry containing 4.3 g (dry) of the yeast described in Example I were mixed with 3.4 g of a 50% (w/w) solution of pirimicarb in xylene for 4 hours at 50°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination of the product showed several globules occupying approximately 80% of the yeast cell. Examination by bio-assay and chemical analyses indicated the presence of encapsulated active insecticide.

Example XIII

30 9.8 g of an aqueous slurry containing 2.1 g (dry) of the yeast described in Example I were mixed with 1.56 g of a 30% (w/w) solution of permethrin in xylene for 16 hours at 45°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination showed several small globules occupying approximately 60% of the yeast cell.

Example XIV

24.0 g of an aqueous slurry containing 4.56 g (dry) of the yeast described in Example I were mixed with 3.65 g of an 80% (w/w) solution of diazinon in xylene for 4 hours at 50°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination showed a large globule occupying the whole of the yeast cell.

Example XV

21.55 g of an aqueous slurry containing 4.4 g (dry) of the yeast described in Example I were mixed with 3.53 g of a 30% (w/w) solution of carbaryl (about 85% active) in xylene for 6 hours at 45°C in a temperature-controlled mixing vessel. The product was harvested as described in Example I.

Microscopic examination showed a large globule in the yeast cell.

Example XVI

24.6 g of an aqueous slurry containing 4.6 g (dry) of the yeast described in Example I were mixed with 3.74 g of a 50% (w/w) solution of resmethrin (about 45% active) in xylene for 6 hours at 45°C in

a temperature-controlled reaction vessel. The product was harvested as described in Example I.

Microscopic examination showed a similar product to that of Example XIII (permethrin).

CLAIMS

1. An encapsulated product comprising a microbial capsule having a lipid content of less than 10% by weight and containing a substantial quantity of an organic liquid, optionally with a material dissolved or microdispersed in the organic liquid, not being natural components of the microbe.
2. An encapsulated product according to claim 1 wherein the microbe has a negligible or substantially nil lipid content.
3. An encapsulated product according to claim 1 or 2 wherein the microbe is a fungus.
4. An encapsulated product according to any of the preceding claims wherein the microbe is a yeast.
5. An encapsulated product according to any of the preceding claims wherein the microbe has a cell size in the range of 5 to 20 microns diameter.
6. An encapsulated product according to any of the preceding claims wherein the microbe is brewers' yeast, bakers' yeast or *Candida utilis*.
7. An encapsulated product according to any of the preceding claims wherein the organic liquid comprises an alcohol having more than 3 carbon atoms.
8. An encapsulated product according to any of the preceding claims wherein the organic liquid comprises an ester.
9. An encapsulated product according to any of the preceding claims wherein the organic liquid comprises a liquid hydrocarbon.
10. An encapsulated product according to any of the preceding claims wherein the organic liquid has dissolved or microdispersed therein one or more dyes suitable for use in carbonless copy paper capsules.
11. An encapsulated product according to any of claims 1 to 9 wherein the organic liquid has dissolved or microdispersed therein an insecticide.
12. An encapsulated product according to claim 11 wherein the insecticide is selected from organochlorine compounds, organo-phosphorus compounds, carbamate compounds and synthetic pyrethroid compounds.
13. An encapsulated product according to claim 1 and substantially as described in any of the foregoing Examples.
14. An encapsulated product according to claim 1 and substantially as described herein.
15. A method of making an encapsulated product comprising contacting a grown microbe having less than 10% by weight lipid content with an organic liquid which is capable of entering the microbe by diffusion through the microbial cell wall without rupturing it, for a contact time being at least until 1 or more glistening globules of the organic liquid can be observed to be retained passively in the microbe.
16. A method according to claim 15 comprising

stirring the microbe with the liquid at a slightly elevated temperature.

17. A method according to claim 16 wherein the temperature is in the range 40°C to 50°C.

5 18. A method according to any of claims 15 to 17 wherein the contact time is at least 2 hours.

19. A method according to any of claims 15 to 18 wherein the microbe is in the form of an aqueous paste or slurry.

10 20. A method according to any of claims 15 to 18 wherein the microbe is in merely moist form.

21. A method according to any of claims 15 to 20 wherein the microbe is treated with a softening agent such as a proteolytic enzyme to soften the
15 cell wall, before or after absorption of the organic liquid.

22. A method according to any of claims 15 to 20 wherein the microbe is treated with a hardening agent such as an aldehyde to harden the cell wall,
20 after absorption of the organic liquid.

23. A method according to claim 15 and substantially as described in any of the foregoing Examples.

24. A method according to claim 15 and sub-
25 stantially as described herein.